Research Methods

Site selection

Locations for large-scale restoration activity were determined using a geographical information system (GIS) based SAV Restoration Targeting System (Parham and Karrh 1998). The model uses six layers of data to evaluate the potential of a particular habitat to support SAV populations. The data layers incorporated into the targeting model included:

1. *Shoreline*: The Maryland shoreline datalayer used was digitized by the Soil Conservation District using United States Geological Survey (USGS) quad sheets at a scale of 1 inch = 24,000 feet.

2. *Water Quality*: The water quality parameter allows site evaluation based on three methods: percent light at leaf, percent light at water (Kemp et al. 1995), or the individual water quality parameters (Dennison et al. 1993). Six water quality parameters important to SAV communities were incorporated into the SAV Restoration Targeting System: light extinction coefficient (Kd), dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorous (DIP), total suspended solids (TSS), chlorophyll a (CHLA), and salinity. Data from a running three year growing season (April to October) for SAV were used to obtain a median value by station for each parameter. The data were obtained from the Chesapeake Bay Mainstem and Tributary Water Quality Monitoring Program as well as several additional spatially targeted water quality programs. The individual water quality parameters were interpolated using the Inverse Distance Weighted interpolation

method in ArcView Spatial Analyst (ESRI) using the four nearest neighbors and 100 foot interpolated cells extending beyond the extent of the Chesapeake Bay. After interpolation of the individual parameters, each parameter was overlayed with salinity coverage and assigned as pass or fail based on the SAV habitat requirements for one meter restoration (Batuik et al. 1992).

3. *Bathymetry*: One and two meter bathymetry contours for the Chesapeake Bay were obtained from the EPA's CBP, intersected with the Soil Conservation District shoreline and converted from lines to polygons. The resulting shapes were designated to yield areas less than one meter depth, areas one to two meters depth, and areas greater than two meters depth at mean low water.

4. *Submerged Aquatic Vegetation*: SAV distribution coverage data was determined based on aerial surveys completed by the Virginia Institute of Marine Science (1981-2004). A composite layer of historical SAV distribution was created by combining the 1981, 1984-1990, and 1991-2004 SAV aerial surveys. Current distribution was based on the 2003-2004 SAV distribution.

5. *Hydraulic Clam Dredging*: Prohibited clamming areas were mapped based on the laws in the Code of Maryland regulating this activity (§4-1037 and §4-1038). MD-DNR natural oyster bar habitats were buffered by 150 feet as called for in the State and County laws. A shoreline setback was established and buffered to the appropriate distance (distance varying by County) using the Soil Conservation District Shoreline coverage.

6. *Blue Crab habitat areas*: These areas were included to encourage placement of SAV restoration activities in areas that are not only suitable for restoration

activities but also provide benefits for species having recognized links to SAV. These areas were defined as outlined in the 1997 Blue Crab Fisheries Management Plan.

A point system is assigned to each data layer and an algorithm developed to evaluate the restoration potential of sites within each tributary based upon the combined datalayers. Figure 3 illustrates a SAV restoration potential map of the Patuxent River based on the SAV Restoration Targeting System.

Study Area

Five sites in the lower Patuxent River; Parrans Hollow (38° 24.714' N 76° 31.649' W), Jefferson Patterson Park (38° 24.438' N 76° 31.268' W), Myrtle Point (38° 19.755' N 76° 29.493' W), Hungerford Creek (38° 20.975' N 76° 28.317' W), and Solomons Island (38° 18.898' N 76° 27.252' W) were identified as suitable habitats for eelgrass recolonization based on the MD-DNR SAV Restoration Targeting System (Figure 4).

Seed Collection

In order to determine the progress of eelgrass seed development and maturation, surveys of reproductive shoot and seed development within potential donor beds in Tangier Sound began in March and continued through April and May of 2004 and 2005. Surveys were conducted by removing a small number of reproductive spadices from plants in possible donor beds and determining the percentage of mature seeds per spadix. Harvesting did not begin until at least 50% of the seeds within reproductive shoot

spadices were mature to ensure harvesting takes place during the peak of seed production. As seed maturation progressed through the spring, self-contained underwater breathing apparatus (SCUBA) was used to conduct surveys to directly compare the density of seeds between potential donor beds. Reproductive shoots were removed from plants in a 1 m^2 sample within a potential donor bed. The total number of seeds per m^2 for a given donor bed was determined as the sum of mature seeds per reproductive spadix, the number of spadices per reproductive shoot, and the number of reproductive shoots in the 1 m^2 samples (Inglis and Waycott 2001).

In 2003, reproductive shoots containing mature seeds were collected manually while snorkeling or using SCUBA over eelgrass beds in Tangier Sound (38° 00.530' N 75° 58.349' W) (Figure 5). During subsequent harvests (2004 and 2005), a mechanical harvest boat (Pristine Marine, M J McCook & Associates, La Plata, MD) was utilized to increase the efficiency and amount of reproductive material collected. In 2004, seeds were collected from donor beds in the Little Annemessex River (37° 58.479' N 75° 52.255' W) and in 2005 from the Little Annemessex River and the mouth of Acre Creek (Big Annemessex River) (37° 59.626' N 75° 51.636' W and 38° 01.718' N 75° 50.632' W, respectively). Immediately following collection, reproductive material was manually loaded into nylon mesh laundry bags, secured at a nearshore dock, and kept submerged in ambient water overnight. Bags of harvested seed material were transported via commercial waterman vessels to the MD-DNR Piney Point Aquaculture facility in St. Mary's County, MD within 24 hours of collection. A portion of the harvested material was transferred to seed bags for immediate deployment (2004), while the seed processing

procedure began on the remaining reproductive material in order to extract mature seeds for fall broadcast (2003, 2004, 2005).

Test Plantings

In 2003, during the site selection process and again in 2004, alongside seed dispersals, test plantings were carried out to ensure that areas identified by the site selection model (Parham and Karrh 1998) would support growth of eelgrass. Adult eelgrass plants were acquired from Tangier Sound in 2003, Tangier Sound and Chincoteague Bay in 2004, and Chincoteague Bay in 2005. A small portion of the total plants used in 2004 were raised from seed at the Piney Point Aquaculture Facility. Plants were transplanted into three 1 m^2 test plots located adjacent to seed broadcast and seed bag areas. A density of 64 adult plants/m^2 was used for each test plot. Bamboo skewers were used to anchor plants in transplant areas.

Seed Bag Deployment

In 2004, a portion of collected eelgrass reproductive seed material was prepared for immediate deployment following a buoy-deployed seeding system (BuDSS) developed by Pickerell et al. with modifications (2003; 2005). A known volume of reproductive material was subsampled and the number of seeds enumerated. Based on the seed counts, a volume of reproductive seed material necessary to achieve the desired seeding density was transferred to pre-measured, coarse (7 mm) mesh bags, buoys added, and bags fastened securely with cable ties (Figure 6). Two sizes of seed bags were constructed: single (5000 seeds or 100,000 seeds/acre) and double (10,000 seeds or 200,000

seeds/acre). Completed seed bags were transported to planting locations at Parrans Hollow, Myrtle Point, and Solomons Island. On location, a predetermined number of seed bags were attached to cinderblock anchors, and deployed in a grid pattern ranging from 6x5 to 11x11 with 10 meters of spacing between bags. Planting density at each location was estimated to be 37 seeds/m^2 despite varied plot size at each location. Details of 2004 seed bag deployments including location, seeding density, areas covered, are presented in Table 1.

Seed Processing, Storage, and Broadcast

Seed Processing

Upon arrival at Piney Point Aquaculture facility, harvested seed material was emptied from mesh laundry bags into one of eight, 20,000 gallon (32'x32'x4') or one of sixteen 9,800 gallon (20'x20'x4') greenhouse basins. The water in each basin was replaced daily with water from nearby St. Georges Creek and augmented with aquaculture grade sea salt to match conditions at the harvesting areas (~14ppt). In addition, each basin was aerated to prevent anoxia and water quality was monitored twice daily. Typical basin dissolved oxygen levels averaged 5-6 mg/l. Basin water quality data is presented in Appendix A. While in the basins, the eelgrass seeds slowly dropped from the reproductive shoots over the following month. After all the seeds had been released and settled to the bottom of the basins, the seed/reproductive shoot slurry was pumped into a series of stacked settling trays to allow the passive accumulation of seeds while discarding the non-seed material.

Seed Storage

Once separated from reproductive material, the seeds were held in a 2500 gallon tank where water was replaced daily, aerated, and augmented with aquaculture grade sea salt until dispersal in October (2004) or August and October (2005). Seed storage water quality data is presented in Appendix B.

Seed Enumeration

Two methods were utilized to enumerate eelgrass seeds. Estimates of the number of seeds collected and utilized in the construction of spring seed bags were made shortly after collection by counting seeds in four 1L replicate subsamples of reproductive material and multiplying the resulting seeds/L by the total volume of harvested material.

In order to count and determine the viability of seeds to be used for fall seed broadcast, water from the storage tanks was drained completely and the total volume of seed material was measured. The total number of seeds were counted from replicate 2 ml samples of seed material. As seeds were being counted, viability was determined using a "squeeze test" (Orth personal communication 2004). This resulted in a total number of seeds and the number of viable seeds (as a percentage of the total counted) per 2 ml sample. The total number of viable seeds was then extrapolated from this based on the total volume of seeds collected. This procedure was repeated just before broadcast to detect any seed loss that may have occurred during storage and for an accurate measurement of viable seeds for calculating broadcast volumes.

Seed Broadcast

Eelgrass seeds were hand (manually) broadcast (Orth et al. 1994) during the fall of 2003 at the Jefferson Patterson Park location (Figure 7A). A density of 100,000 seeds/acre was used across a total of six 0.5 acre circles (three total acres = 300,000 total seeds). To ensure uniform seed distribution, each of the six circles were divided into 5 m rings. Each ring represented a percent of the total area of the circle. That proportion was used to determine the proportion of seed (50,000 per ring) to be broadcast in that given area (Figure 8). A metal pipe was anchored in the center of the circle and a rope marked at 5 m intervals was attached. Two biologists broadcast proportioned seed aliquots in tandem around one 5 m ring at a time until each ring had been broadcast. Table 2 provides details of the manual seed broadcast at Jefferson Patterson Park in 2003.

A mechanical seed sprayer, mounted to a boat, capable of evenly dispersing seeds at suitable densities (100,000 to 300,000 seeds/acre) at the rate of 10 minutes/acre (C& K Lord, Inc) (Figure 7b), was utilized to broadcast seeds in the Fall of 2004 at the Hungerford Creek, Parrans Hollow, and Solomons Island locations and in 2005 at Jefferson Patterson Park, Hungerford Creek, and Myrtle Point locations. The area of bottom to be planted was multiplied by the desired planting density to determine the total number of seeds necessary. The volume of seeds needed to achieve the desired seeding density was determined based on the percent of viable seeds of the total volume (Orth, personal communication 2004). The flow of the seed sprayer mechanism was then calibrated and adjusted to distribute seeds uniformly at the desired density. Seeds were loaded into the seed broadcast machine and expelled into the water column. All seed broadcasts took place in October before the ambient water temperatures dropped below

15°C, when eelgrass seed germination begins (mid-November to December) (Orth and Moore 1983; Moore et al. 1993). Table 3 provides details of the 2004 and 2005 mechanical seed broadcasts in the Patuxent River.

Surveying and Monitoring

Site Surveys

The survivability of the transplanted, adult, eelgrass plants within test plot areas was evaluated by determining presence or absence of adult plants within the three 1 m^2 test plots at seven months, nine months, and thirteen months after initial planting (May, July, and November). Assessment sof seedling abundance in fall seed broadcast and spring seed bag areas were made beginning in May 2005, seven months after seeding, and the beginning of the eelgrass spring growing season. The eelgrass seedlings were enumerated along two or three non-destructive, 1 m^2 belt transects (Burdick and Kendrick 2001). Using SCUBA, divers completed transects diagonally across the study plots from an offshore corner to the opposite inshore corner using compass bearings. The total number of seedlings along the 1 m^2 transects was then used to extrapolate the number of seedlings present throughout the area (m^2) of the entire seeded plot. Initial planting success was then determined by comparing the total number of seedlings observed to the total number of seeds dispersed in the plot. This same method was repeated in July and November (nine and thirteen months after seeding, respectively) to determine the persistence of seedlings.

The initial planting success was calculated as the proportion of the total number of seeds dispersed that became established (# of seedlings in the sediment confirmed by divers/total number of seeds dispersed) during the first survey (May 2005). The survival of plants was calculated as the proportion of initially established seedlings or adult plants that persisted after nine (July 2005) and thirteen months (November 2005).

Water Quality Monitoring

Spatially intensive water quality monitoring (water quality mapping) was conducted monthly throughout the eelgrass growing season (March - November) throughout the lower portion of the Patuxent River utilizing MD-DNR DATAFLOW systems. DATAFLOW is a shipboard system of geospatial equipment and water quality probes that measure water quality parameters from a flow-through stream of water collected near the water's surface (Madden and Day 1992). Five water quality parameters (water temperature, salinity, dissolved oxygen, turbidity in nephelometric turbidity units (NTU), and fluorescence) were measured. Each water quality datum is associated with a date, time, water depth, and GPS coordinate (NAD83) reported to six decimal places.

Two continuous monitoring (YSI 6600 EDS) stations were located on the Patuxent River prior to and during restoration (2003-2005) to provide temporally intensive habitat assessments to complement the monthly water quality mapping. The first monitoring station was located at the Pin Oak Farm (38° 24.528' N 76° 31.308' W), near the Parrans Hollow and Jefferson Patterson Park restoration sites and the second monitoring station at the Chesapeake Biological Laboratory dock (38° 19.002' N 76° 27.156' W), near the

Solomons Island restoration site. Each continuous monitor recorded seven water quality parameters (water temperature, salinity, dissolved oxygen, turbidity, fluorescence, pH, and reduction potential) every 15 minutes. Both meters were located at a constant depth of approximately one meter below the surface of the water. The continuous monitors were deployed throughout the SAV growing season and data was downloaded weekly during deployment.

Fixed station water quality monitoring cruises were conducted monthly at eleven stations throughout the mainstem of the Patuxent River beginning in 1985 and continuing through 2005.

Detailed information for the Maryland Department of Natural Resources Chesapeake Bay Shallow Water Quality Monitoring Program, including specific methods of the DATAFLOW, Continuous Monitoring, and Mainstem Cruise Programs, can be accessed:
2004: http://mddnr.chesapeakebay.net/eyesonthebay/swm_qapp_2004.pdf
2005: http://mddnr.chesapeakebay.net/eyesonthebay/swm_qapp_2005.pdf

Cost Per Acre and Survival Calculations
At the conclusion of the first year of restoration, several calculations were made. To determine the financial investment made per seed dispersed, the total cost of the particular method was divided by the total number of viable seeds dispersed using that method.

Cost per seed = Total cost associated with method/Total number of viable seeds dispersed

The total cost for restoring one acre could then be calculated by multiplying the cost per seed by the specified seeding density (200,000 seeds/acre). The recruitment success of each method was determined by dividing the total number of seeds dispersed by the number of successfully recruited plants. The total cost for each method was divided by the total number of successfully recruited seedlings to determine a ratio of cost per successfully recruited seedling between the spring seed bag and fall seed dispersal methods. For the purpose of cost comparison between methods, site selection and water quality monitoring costs were not included.